CLEAN VERSION WITH CHANGES

1. A method of isolating RNA comprising an oligo- or polynucleotide from a sample comprising the steps of:

(a) treating the sample with a reactant capable of covalently modifying the 2'-OH position of the ribose rings of the RNA under conditions so that a proportion of the 2'-OH positions of the ribose rings bear a substituent; and

(b) preparing isolated RNA therefrom by separating material containing the substituent from the sample on the basis of a property of the substituent.

2. The method according to claim 1, wherein:

(a) step (a) of Claim 1 is carried out in a reaction medium which comprises an organic solvent, and optionally wherein said organic solvent comprises an organic base, and further optionally wherein said reactant comprises an acid anhydride, an acid chloride, a carboxylic acid or an N-acylimidazole, and further optionally wherein said reaction medium further comprises an acylation catalyst, and further optionally wherein said the reaction medium further comprises water;

(b) said RNA comprises mRNA, rRNA or viral RNA;

(c) said sample comprises a sample from a biological source;

(d) said sample includes DNA;

(e) said substituent comprises a solid phase, and optionally wherein said solid phase comprises benzoyl chloride polymer bound (BCPB) beads, silica particles or particles of a glass, and further optionally wherein said solid phase is modified to introduce a reactive group which reactive group is capable of reacting with RNA to capture the RNA on the solid phase, and further optionally wherein said reactive

group is introduced by modifying the solid phase with a bi-functional acid halide;

(f) said substituent comprises a hydrophobic substituent, and optionally wherein said hydrophobic substituent comprises a substituent, OR, wherein R is selected from the group consisting of: C_1-C_{36} alkyl; C_1-C_{36} alkenyl; C_1-C_{36} alkynyl; C_1-C_{36} haloalkyl; C_1-C_{36} aminoalkyl; C_1-C_{36} alkoxyalkyl; C_1-C_{36} alkylthioalkyl; C_1-C_{36} alkoxyalkoxyalkyl; C_1-C_{36} haloalkoxyalkyl; C_1-C_{36} aminoalkoxyalkyl; C_6-C_{36} aryl; C_6-C_{36} alkylaryl; C_6-C_{36} arylalkyl; C_6-C_{36} arylalkenyl; C_1-C_{36} alkanoyl; C_1-C_{36} alkenoyl; C_1-C_{36} haloalkenoyl; C_1-C_{36} haloalkanoyl; C_2-C_{36} haloformylalkanoyl; C_1-C_{36} C_1-C_{36} aminoalkanoyl; C_1-C_{36} azidoalkanoyl; C_1-C_{36} carboxyalkanoyl; C_1-C_{36} carboxyalkenoyl; C_1-C_{36} carboxyalkynoyl; C_1-C_{36} alkylaminoarylalkanoyl; C_1-C_{36} alkoxycarbonyl; C_1-C_{36} alkenyloxycarbonyl; C_1-C_{36} alkylsulfonyl; C_6-C_{36} arylalkanoyl; C_6-C_{36} arylalkenoyl; C_6-C_{36} aryloxyalkanoyl; C_6-C_{36} alkylarylalkanoyl; C_6-C_{36} haloarylalkanoyl; C_6-C_{36} aminoarylalkanoyl; C_1-C_{36} alkylsilanyl; C_1-C_{36} trialkylsilanyl and C_{12}-C_{28} diarylphosphano; or a substituent R', wherein R' comprises C_1-C_{36} alkyl; C_1-C_{36} alkenyl; C_1-C_{36} alkynyl; C_1-C_{36} haloalkyl; C_1-C_{36} aminoalkyl; halo; amino; C_1-C_{36} alkylamino; C_6-C_{36} aryl; C_1-C_{36} alkylaryl or C_1-C_{36} arylalkyl;

(g) said hydrophobic substituent of (f) comprises a C_4 to C_7 carbon chain or ring;

(h) wherein said reactant comprises butyric anhydride, pentanoic anhydride, hexanoic anhydride or benzoic anhydride;

(i) said proportion of 2'-OH positions bearing the substituent is at least 10%;

(j) said hydrophobic substituent of (f) comprises a C_8-C_{12} carbon chain or ring, and optionally wherein said proportion of 2'-OH positions bearing the substituent is in the range 1 to 10%;

(k) said hydrophobic substituent of (f) comprises a C_{12}-C_{36} carbon chain or ring, and optionally wherein said proportion of 2'-OH positions bearing the substituent is up to 1%;

(l) said step (b) comprises contacting the treated sample from step (a) with a hydrophobic solid phase so as to bind the material containing the hydrophobic substituent and optionally washing the material bound to the solid phase, and optionally wherein said hydrophobic solid phase comprises hydrophobic particles, and further optionally wherein said method further comprises a step of eluting the material bound to the hydrophobic solid phase by treating with a detergent, a chaotrope or a solvent, by lowering the salt concentration or by cleaving the substituent from the 2'-OH position of the ribose rings;

(m) said step (b) comprises the further step of treating the treated sample from step (a) with a lyotrophic salt to aggregate the material containing the hydrophobic substituent as an RNA precipitate, and isolating the precipitate, and optionally wherein said lyotrophic salt comprises ammonium sulphate, an alkali metal chloride, magnesium chloride or calcium chloride; or

(n) said step (b) comprises treating the treated sample with a non-polar solvent to form a hydrophobic liquid phase which contains the material containing the hydrophobic substituent, and isolating the hydrophobic liquid phase, and optionally wherein said non-polar solvent comprises pentane, cyclohexane, toluene, benzene, light petroleum, xylene or hexane.

3. A kit for the preparative isolation of RNA comprising an oligo- or polynucleotide from a sample, which kit comprises:

(i) a reaction system for modifying the RNA to form a modified oligo- or poly-nucleotide in which a proportion of the 2'-OH positions of the ribose rings bear a substituent; and

(ii) a separation system for preparing isolated RNA by separating material containing the substituent from the sample on the basis of a property of the substituent.

4. The kit according to Claim 3, wherein said reaction system comprises:

(a) an organic solvent; and

(b) a reactant capable of covalently modifying the 2'-OH position of the ribose rings of the RNA in the presence of the organic solvent, and optionally wherein:

(i) said organic solvent comprises an organic base;

(ii) said reactant comprises an acid anhydride, an acid chloride, a carboxylic acid or an N-acylimidazole;

(iii) said kit comprises an acylation catalyst;

(iv) said substituent comprises a solid phase, and optionally wherein said solid phase comprises benzoyl chloride polymer bound (BCPB) beads, silica particles or particles of a glass; and optionally wherein:

(c) said substituent comprises a hydrophobic substituent, or more specifically wherein said hydrophobic substituent comprises a substituent, OR, wherein R comprises a moiety selected from the group consisting of: C_1-C_{36} alkyl; C_1-C_{36} alkenyl; C_1-C_{36} alkynyl; C_1-C_{36} haloalkyl; C_1-C_{36} aminoalkyl; C_1-C_{36} alkoxyalkyl; C_1-C_{36} alkylthioalkyl; C_1-C_{36} alkoxyalkoxyalkyl; C_1-C_{36} haloalkoxyalkyl; C_1-C_{36} aminoalkoxyalkyl; C_6-C_{36} aryl; C_6-C_{36} alkylaryl; C_6-C_{36} arylalkyl; C_6-C_{36} arylalkenyl; C_1-C_{36} alkanoyl; C_1-C_{36} alkenoyl; C_1-C_{36} haloalkenoyl; C_1-C_{36} haloalkanoyl; C_2-C_{36} haloformylalkanoyl; C_1-C_{36} C_1-C_{36} aminoalkanoyl; C_1-C_{36} azidoalkanoyl; C_1-C_{36} carboxyalkanoyl; C_1-C_{36} carboxyalkenoyl; C_1-C_{36} carboxyalkynoyl; C_1-C_{36} alkylaminoarylalkanoyl; C_1-C_{36} alkoxycarbonyl; C_1-C_{36} alkenyloxycarbonyl; C_1-C_{36} alkylsulfonyl; C_6-C_{36} arylalkanoyl; C_6-C_{36} arylalkenoyl; C_6-C_{36} aryloxyalkanoyl; C_6-C_{36} alkylarylalkanoyl;

(j) said separation system comprises a non-polar solvent for forming a hydrophobic liquid phase which contains the material containing the hydrophobic substituent.

6. A preparative device for isolating RNA comprising an oligo-or polynucleotide from a sample from a subject, which device comprises:

(i) a means for extracting the sample from the subject;

(ii) a reaction system for modifying RNA in the sample to form a modified oligo- or poly-nucleotide in which a proportion of the 2'-OH positions of the ribose rings bear a substituent; and

(iii) a separation system for preparing isolated RNA by separating material containing the substituent from the sample on the basis of a property of the substituent.

7. The device according to claim 6, wherein:

(a) said means for extracting the sample from the subject comprises a syringe needle;

(b) said substituent comprises a solid phase, and optionally wherein the solid phase comprises a membrane, a particle, a bead, a filter, a fibre, a gel, a strip, a matrix, a resin, a capillary or the walls of a vessel;

(c) said sample comprises biological material; or

(d) said device further comprises a filter for removing red and/or white blood cells.

C_6-C_{36} haloarylalkanoyl; C_6-C_{36} aminoarylalkanoyl; C_1-C_{36} alkylsilanyl; C_1-C_{36} trialkylsilanyl and C_{12}-C_{28} diarylphosphano; or a substituent R', wherein R' comprises C_1-C_{36} alkyl; C_1-C_{36} alkenyl; C_1-C_{36} alkynyl; C_1-C_{36} haloalkyl; C_1-C_{36} aminoalkyl; halo; amino; C_1-C_{36} alkylamino; C_6-C_{36} aryl; C_1-C_{36} alkylaryl or C_1-C_{36} arylalkyl.

5. The kit according to claim 4, wherein:

(a) said hydrophobic substituent comprises a C_4 to C_7 carbon chain or ring;

(b) said reactant comprises butyric anhydride, pentanoic anhydride, hexanoic anhydride or benzoic anhydride;

(c) said proportion of 2'-OH positions bearing the substituent is at least 10%;

(d) said hydrophobic substituent comprises a C_8-C_{12} carbon chain or ring;

(e) said proportion of 2'-OH positions bearing the substituent is selected from any one integer from 1 to 10% inclusive;

(f) said hydrophobic substituent comprises a C_{12}-C_{36} carbon chain or ring;

(g) said proportion of 2'-OH positions bearing the substituent is up to 1%;

(h) said separation system comprises a hydrophobic solid phase for binding the material containing the substituent, and optionally wherein said hydrophobic solid phase comprises hydrophobic particles, and further optionally wherein said separation system further comprises an elution medium for eluting RNA bound to the hydrophobic sclid phase;

(i) said separation system comprises a lyotrophic salt for aggregating the material containing the hydrophobic substituent; or